Predator and Environmental Effects on the Polymorphic Egg Masses of Spotted Salamanders (*Ambystoma maculatum*)

by

Simon Leslie King

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Approved by:

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Lynn Siefferman, Ph. D, Thesis Director

_____

Michael Osbourn, Ph. D., Second Reader

_____

Lynn Siefferman, Ph. D, Departmental Honors Director

Abstract

Polymorphisms may be maintained if selection intensity and gene flow vary across a species' geographic range. Jelly coats of amphibian eggs are under many different selective forces, such as predators and external environment interactions. Spotted salamanders (*Ambystoma maculatum*) have polymorphic egg masses that are either clear or opaque depending on the presence or absence of hydrophobic protein crystals in the outer egg layer. This study investigated how different predator communities and environmental parameters influence the distribution of the polymorphic egg masses in high and low elevations of North Carolina. I conducted surveys of *A. maculatum* clutches in breeding ponds and recorded numbers of clear and opaque egg masses, as well as the presence of predator and water chemistry in seven North Carolina counties. I found that egg masses at high elevation sites were predominately opaque (~82%), whereas egg masses at low elevation sites were predominately clear (~98%). Although water chemistry (pH, conductance) varied greatly between high and low elevation locations, water chemistry was correlated with egg polymorphism only in the mountains. At both elevations, locations with greater predator occupancy tended to have higher proportions of opaque egg masses. These results suggest the selective forces shaping the distribution of *A. maculatum* egg masses include both predator and physiochemical forces, but the additive effects of both stressors may drive the high ratios of opaque egg masses.

Introduction

Polymorphisms may be maintained if selection intensity and gene flow vary across a species' geographic range (Thompson, 2005). Jelly coats of amphibian eggs are under many different selective forces, including predators (Altig & McDiarmid, 2007) and physiochemical environmental conditions (Shu et al., 2015). Variation in sources of selection pressure may influence the advantage or disadvantage of different morphs due to spatial differences in predator preference (Blanco & Bertellotti, 2002) or in physical environmental variables (Ruth et al. 1993; Pintar & Resetarits, unpublished data). Further, if a predator's influence differs spatially over the prey's geographic range, this can lead to location-specific evolved defense mechanisms in the prey (Hochberg & van Baalen, 1998). Different phenotypic traits are commonly used as defense mechanisms and predation is important in shaping the distribution of these traits (Stoks et al., 1999).

Reproduction is a costly event for amphibians, with some salamander species investing up to 48% of their annual metabolic energy on reproduction, two-thirds of which goes to egg production (Fitzpatrick, 1973). Amphibians have adapted to lay the optimal number and size of eggs depending on environmental conditions to maximize their lifetime fitness (Wilbur, 1977). Egg polymorphism in animals can result from differential selection for alleles that enhance egg survival. The degree of selection can depend on the magnitude of selective pressure based on predation (Blanco & Bertelotti, 2002) and environmental conditions (Shu et al., 2015b).

Spotted salamanders (*Ambystoma maculatum*) occur in most of eastern North America and reproduce during early spring in wetlands. They, like many amphibians, have adapted to produce eggs with a thick jelly around them, which functions as a layer to protect

developing embryos from predators and other environmental variables (Altig & McDiarmid, 2007). A symbiotic relationship between a green algae (*Oophila amblystomatis*) and *A. maculatum* has also evolved, in which *O. amblystomatis* supplements dissolved oxygen levels experienced by developing *A. maculatum*, thereby enhancing growth rates (Pinder & Friet, 1994). *Ambystoma maculatum* egg masses have two distinct color morphs: clear and opaque. The dimorphic coloration is caused by the presence of hydrophobic protein crystals in the outer layer of opaque egg masses, whereas clear egg masses lack this and instead have a water-soluble protein (Hardy & Lucas, 1991). Ruth et al. (1993) determined that egg mass color was due to a simple polymorphism of a single gene. Additionally, other support for a genetic basis includes: (a) females in captivity never lay more than one egg mass type over successive years, (b) egg mass types are often sympatric in ponds suggesting environmental conditions are not the immediate cause, and (c) females that lay opaque egg masses have white ovisacs, while females that lay clear egg masses have black ovisacs (Hardy & Lucas, 1991). Within populations, egg dimorphism can vary substantially, and may range 0 to 100% of either color mass (Ruth et al., 1993). Previous studies have found ponds in central Pennsylvania (Ruth et al., 1993) and western North Carolina (Petranka, unpublished data) had higher proportions of opaque egg masses, while ponds in northern Mississippi had higher proportions of clear egg masses (Pintar & Resetarits, unpublished data). If this dimorphic trait is genetic and varies with geography, then there is likely spatial variation in the relative advantages of each egg mass type.

Numerous taxa are known predators of *A. maculatum* egg masses, including caddisflies (families Limnephilidae and Phryganeidae) (Stout et al., 1992), leeches (Cargo, 1960), marbled salamanders (*Ambystoma opacum*) (author, pers. obsv.), fish (Semlitsch,

1988), crayfish (Ward & Sexton, 1981), spotted turtles (*Clemmys guttata*) (Tyning et al., 1990), eastern newts (*Notophthalmus viridescens*) (Hamilton, 1932), and wood frogs (*Lithobates sylvaticus*) (Petranka et al., 1998). Stout et al. (1992) found that larval caddisflies were capable of inflicting significant mortality on *A. maculatum* eggs, and that caddisflies found on egg masses were larger than individuals found on the benthos. Rowe et al. (1994) found a significant effect of caddisfly predation on overall hatching success of *A. maculatum*, but there was no difference in predation between clear and opaque eggs.

In the southern Appalachian Mountains, *L. sylvaticus* may be an important predator of *A. maculatum* clutches. While *L. sylvaticus* are primarily microphagous filter feeders, they opportunistically feed upon the eggs and hatchlings of *A. maculatum* (Petranka et al., 1998). Predation of *L. sylvaticus* tadpoles upon *A. maculatum* egg masses results in reduced egg mass size, leaving embryos exposed and vulnerable to tadpole attack and even death (Petranka et al., 1998). Previous studies have demonstrated *L. sylvaticus* are the primary egg predators of *A. maculatum* in sympatric breeding ponds (Petranka et al., 1998) and the tadpoles predate clear egg masses more frequently than opaque egg masses (Petranka et al. 1998; Jacobson, 2015). Further, Petranka et al. (1998) found the proportion of opaque egg masses increased significantly with the egg density of *L. sylvaticus*. Another study has demonstrated that *L. sylvaticus* presence at high densities strongly decreased larval survival, growth, and development of *A. maculatum* (Holbrook & Petranka, 2004). While *L. sylvaticus* is the only predator in which a preference for morphs has been documented, other *A. maculatum* egg predators may also drive selection for a particular morph.

In addition to predation, physical and chemical attributes of breeding ponds may also influence the evolution of egg morphology and function. Amphibian egg jellies can protect

embryos from contaminants (Marquis et al., 2006), oomycete infections (Urban et al., 2015), desiccation (Marco & Blaustein, 1998), and water molds (Gomez-Mestre et al., 2006). Egg masses also help insulate and accumulate heat for developing embryos (Beattie, 1980). Urban et al. (2015) investigated oomycete infection resistance capabilities in *A. maculatum* egg masses, but did not find any difference between clear and opaque egg masses. Ruth et al. (1993) found a relationship between the proportion of clear egg masses with particular cations: [K], [Na], [Ca], and [Mg] and suggested that larval development in clear egg masses might be influenced by these cations.

Dissolved nutrients may influence selection for egg mass color ratios as well; Pintar and Resetarits (unpublished data) found that larvae which emerged from opaque egg masses were larger in low nutrient environments and this relationship persisted throughout the larval stage in Mississippi ponds. Larval survival was also greater for individuals from opaque masses compared to clear, but there was no effect of nutrients on survival (Pintar & Resetarits, unpublished data). These results suggest that opaque egg masses may be advantageous in low nutrient environments, while clear egg masses may be advantageous in high nutrient environments. Further, Pintar and Resetarits (unpublished data) found negative correlations between pond conductivity and proportions of opaque eggs present, where ponds with lower conductivity had higher proportions of opaque masses. Pintar and Resetarits (unpublished data) suggest that the firmer consistency of opaque egg masses might inhibit leaching during nutrient imbalance and therefore reduce degradation that occurs of more pliable clear masses.

It is likely that complex interactions between environmental factors and predators have driven selection for characteristics of *A. maculatum* egg masses (Altig & McDiarmid,

2007). In this study, I test whether varying predator presence and water chemistry influences clear and opaque egg mass ratios of *A. maculatum* in high elevation (mountain) and low elevation (piedmont) ponds in North Carolina. I hypothesize that water chemistry and predator community differences between the two elevations influence the distribution of clear and opaque egg masses. I predict higher ratios of opaque to clear egg masses in high predation locations. Moreover, I predict that water chemistry may influence egg mass ratios and expect that low nutrient areas will have higher ratios of opaque to clear egg masses.

Methods

Study Sites and Species

I conducted surveys between January 30th and April 25th, 2016 across North Carolina in the counties of Avery, Durham, Franklin, Mecklenburg, Orange, Randolph, and Watauga (Figure 1). Sites were chosen based on their location being either within the Piedmont (<450m) or Mountain (>450m) region of North Carolina in order to represent the environmental and predator variation between the high and low elevations. Most sites were selected from historical observations of *A. maculatum* (van Devender, pers. comm.; Dorcas, unpublished data; Howard, unpublished data), whereas others were found during searches for new sites. Twelve sites within low elevations were used and ten sites within high elevations were used. The number of individual ponds varied among sites.

Ambystoma maculatum typically breeds from January through mid-March in fish-free lentic habitats. Female *A. maculatum* attach egg masses to support structures in ponds such as fallen tree branches. Masses are typically 5-15 cm wide, 5-25 cm long, and often have 50-80 eggs in them. Females may deposit 2-4 masses (Petranka et al., 1998).

Experimental Design and Procedure

During surveys, I exhaustively searched for *A. maculatum* egg masses in breeding pools. Search efforts were recorded as number of people searching and amount of time searched. I recorded the number and frequency of clear and opaque egg masses (Fig. 2). At each site, I conducted 15 person-minute dip-net searches for predators and recorded presence/absence for the site. Predators detected were: Eastern newt (*Notophthalmus viridescens*), wood frog tadpoles (*Lithobates sylvaticus*), marbled salamander larvae (*Ambystoma opacum*), caddisfly larvae (family Limnephilidae), fish and crayfish. Elevation was recorded at each site to distinguish geographic regions: our high elevation (mountain) sites ranged from 944-1061 m and Piedmont sites ranged from 59-241 m. I measured water conductivity (SPC), pH, and NO_3 with a YSI Professional Series Plus Multiparameter Instrument W14-04.

Statistical Analysis

I analyzed data using SPSS v.22 (IBM 2015). I ran Generalized Linear Mixed Models and used Akaike information criterion (AIC) to determine the best-fit models for both predators and water chemistry. Two sites at lower elevations were identified as outliers and were not included in the statistical tests. I utilized a Mann-Whitney Test to assess differences in the proportion of clear egg mass between high and low elevations. In all models, the proportion of clear egg masses per pond was the dependent variable and site was the random effect. Predators, pH, NO_3, SPC, and elevation were independent variables. Predator data was classified as present (=1) or absent (=0). Geographic range was categorized based on

elevation with high elevations (mountains) being between 944 and 1061 m, and low elevations (piedmont) being between 59 and 241 m.

Results

I found a significant difference between the proportion of clear egg masses at high and low elevation field sites; lower elevations had a much higher proportion of clear egg masses (98%, $U = 18.0$, $n = 40$, $p < 0.001$; Fig. 3). In the high elevations 18% of egg masses were clear, while at the low elevation sites 98% of egg masses were clear. Predator communities varied between high and low elevations. Predators detected within the high elevations included: *N. viridescens*, caddisfly larvae, *L. sylvaticus* tadpoles, crayfish, and fish. Predators found within the low elevations were *N. viridescens*, *A. opacum*, and crayfish. Only *N. viridescens* and crayfish were found at both high and low elevation sites. The proportion of ponds occupied by *N. viridescens* varied with elevation ($X^2 = 4.31$, $p = 0.038$, $n = 40$). At low elevations, 22% of sites had *N.* viridescens, whereas 54% of high elevation sites had *N. viridescens*. SPC and pH were significantly higher at high elevation ponds compared to low elevation ponds, and NO_3^- did not vary significantly with elevation (Table 1 & Table 2).

High Elevation Sites

In high elevation ponds, there was a significant association between the proportion of clear egg masses and pH (Figure 4) and NO_3^- (Figure 5) (Table 3). While high elevation ponds had higher pH and NO_3^- compared to low elevation sites, ponds in high elevations with lower pH and with lower NO_3 were associated with fewer clear egg masses (Fig 4, Fig 5, Table 3). There was not a significant association between SPC and the proportion of clear

egg masses. The best predator model was *N. viridescens* and I found a significant association between *N. viridescens* presence and the proportion of clear egg masses (Figure 6; Table 5), followed by caddisfly larvae (Figure 7; Table 5). For both models, ponds with either predator present were associated with lower proportions of clear egg masses. There was no significant association between proportion of clear egg masses and presence of crayfish, fish, or *L. sylvaticus* (Table 5).

Low Elevation Sites

In low elevation ponds, there were no significant associations between any measures of water chemistry variables and the proportion of clear egg masses (Table 4). The best-fit predator models to predict egg mass color ratios were *N. viridescens* and *A. opacum*. The proportion of clear egg masses was significantly lower in ponds in which either *A. opacum* (Figure 8; Table 6) or *N. viridescens* (Figure 9; Table 6) were present. Crayfish did not have a significant association with the proportion of clear egg masses (Table 6); no fish or caddisfly larvae were detected in low elevation ponds.

Discussion

Egg mass color ratios differed between high and low elevation field sites; in high elevation sites, on average, 18.7% of egg masses per pond were clear, while at low elevation sites 98% of the masses per pond were clear. Moreover, water chemistry and predator communities differed greatly between elevations; high elevations had significantly higher pH and conductivity (SPC) compared to low elevation sites. These trends suggest that varying selection pressure between the two elevations may be selecting for one morph over the other.

It seems likely that predator communities select for opaque eggs at both elevations. However, only in the mountains does it appear that water chemistry influences clutch polymorphism as well. The higher pH and SPC levels in the mountains may explain the overall higher proportion of opaque egg masses.

Higher pH and SPC levels in the high elevations may be a consequence of earth and atmospheric chemistry, as some of the most important influencing mechanisms that control water chemistry are underlying bedrock composition and atmospheric precipitation (Gibbs, 1970). The high elevation sites are in the Blue Ridge Mountains, which are composed primarily of sedimentary, volcanic, igneous, and metamorphic rocks. Low elevation sites are within the Piedmont, where the geology changes to predominantly metamorphic and igneous rocks (Vocci & Templeton, 2006). The soil in the Mountains and Piedmont regions are also quite different, as it is dependent on the underlying rock types, type of vegetation, and climate (Vocci & Templeton, 2006).

In the high elevations, ponds with a lower pH had lower proportions of clear egg masses, and the proportions increased as the pH levels increased. Despite this trend, the proportion of clear egg masses in high elevations was low, and pH was higher overall compared to low elevations. This may indicate there is an optimal pH range for opaque egg masses to be advantageous. Ruth et al. (1993) reported no apparent association between low pH levels and frequency of clear or opaque egg masses at their central Pennsylvania site. Pintar and Resetarits (unpublished data) show that ponds in northern Mississippi with high nutrients have significantly higher conductivity and lower pH levels, and clear egg masses dominate. My data corroborate these findings as I found a similar relationship between pH and egg mass ratios. Environments with low pH levels are stressful and are associated with

reductions in larval anuran body size (Pakkasmaa et al., 2003). It may be that opaque egg masses confer fitness benefits to clutches in higher pH environments. Shu et al. (2015b) have shown amphibians can alter the morphology of their eggs in environments with varying acidity levels to increase hatching success.

I also found a positive association between NO_3^- concentration and the proportion of clear egg masses in the high elevation sites. Symbiotic interactions abound between *A. maculatum* and *O. amblystomatis* and may be involved in this association. Nitrogenous compounds, such as nitrate (NO_3^-) may limit *O. amblystomatis* growth during *A. maculatum* embryonic development. *Ambystoma maculatum* embryos provide some nitrogenous waste products to *O. amblystomatis* (Small et al., 2014). In low productivity ponds, where *O. amblystomatis* may not receive enough N compounds, the NO_3^- levels in the water might be necessary additional nitrogen for it. Moreover, Ruth et al. (1993) suggest that the less transparent nature of the opaque egg masses may limit algal photosynthetic activity of *O. amblystomatis* and therefore the quality of the symbiosis. Kerney (2011) found algal density increases *A. maculatum* development. If this is the case, then the opaque egg masses may be advantageous in environments with more NO_3^- to assist *O. amblystomatis* growth and productivity as light might be a limiting factor within opaque egg masses (Altig & McDiarmid, 2007). Additionally, Ruth et al. (1993) found both positive and negative correlations between egg mass polymorphisms and pond cations including: K, Na, Ca, and Mg and suggested that larval development in clear egg masses may be influenced by these cations. Future work should use both correlational data and experimental approaches to understand how NO_3^- levels and cations influence both egg mass polymorphisms and *A. maculatum* fitness.

In both high and low elevation field sites, predator presence was significantly associated with a decreased proportion of clear egg masses. In high elevation ponds, both the presence of *N. viridescens* and caddisfly larvae were associated with lower proportions of clear egg masses. Similarly, in low elevation ponds, the presence of *N. viridescens* and *A. opacum* larvae were associated with lower proportions of clear egg masses. These results lend support to the idea that opaque egg masses may better protect eggs from depredation. Altig and McDiarmid (2007) suggest opaque egg masses may help conceal embryos from predators as they are sometimes not visible through the opaque egg masses. Additionally, the hydrophobic proteins present in the outer layer of opaque egg masses, which are not found in clear egg masses, may be unpalatable to predators. The research on *A. maculatum* egg mass palatability is equivocal, as previous studies have indicated that *A. maculatum* egg masses are unpalatable (Stout et al., 1992), while others have indicated they are palatable (Rowe et al., 1994).

My initial interest in this study was driven by the previous studies in the mountains of North Carolina by Petranka et al. (1998) and Jacobson (2015), which demonstrated that *L. sylvaticus* tadpoles preferentially consume clear compared to opaque egg masses. Moreover, Petranka et al. (1998) found a positive correlation between *L. sylvaticus* abundance and proportion of opaque masses. Thus, I expected that sites with *L. sylvaticus* present would have higher ratios of opaque egg masses to counteract predation, but my correlative data showed no such relationship. However, the Petranka et al. (1998) study was limited to a 94-hectare single study site, which likely meant only a few *A. maculatum* populations could occur in such an area. In my study, all of the high elevation (mountain) sites were within the *L. sylvaticus* range, but not all ponds had *L. sylvaticus* present. In both the Petranka et al.

(1998) and Jacobson (2015) studies, the selective advantage of opaque masses occurred when *L. sylvaticus* had no food available except *A. maculatum* clutches and this would increased the likelihood of *L. sylvaticus* preying upon egg masses. Additionally, Petranka et al. (1998) suggest *L. sylvaticus* tadpoles switch to egg masses of *A. maculatum* only when high quality food resources are scarce. It may be that predation by *L. sylvaticus* influences egg mass ratios only if there are few other food options in the breeding ponds and this would seem most likely in systems where high densities of *L. sylvaticus* tadpoles occur.

Notophthalmus viridescens was the only predator detected in both high and low elevation ponds that had a significant association with proportion of clear egg masses, which allows a regional comparison of its potential predatory effect on egg mass ratios. Interestingly, even though *N. viridescens* presence was associated with lower proportions of clear egg masses in low elevations, the proportion of clear egg masses in the whole region was still 98%, whereas the proportion of clear egg masses in high elevations was 18%. Although these results suggest *N. viridescens* might be a selective force for opaque egg masses, *N. viridescens* presence alone does not seem to have the power to explain the drastic difference between egg mass ratios in the high and low elevations. Egg mass color is influenced by many different selective forces, such as predators (Altig & McDiarmid, 2007) and environmental conditions (Shu et al., 2015), and thus has the need respond to various pressures at once.

Another potential advantage of opaque egg masses is they may contain more eggs. Brodman (1995) studied *A. maculatum* clutches in Ohio and found opaque eggs masses contained significantly more eggs than clear egg masses, but the egg morphs did not affect hatching success or length of incubation period. Additionally, Pintar and Resetarits

(unpublished data) found that larvae from opaque egg masses were larger than larvae from clear egg masses in low nutrient conditions and the size difference remained throughout development. Larvae from both opaque and clear egg masses were larger in high nutrient environments compared to low nutrient environments. These results suggest an advantage of opaque egg masses in low nutrient conditions. Larger salamander larvae typically have higher survival, fitness, and will outcompete smaller individuals (Semlitcsch et al., 1998). If opaque egg masses are found in environments with low nutrients and more predators, then having more and larger offspring would be beneficial because more offspring would likely increase the amount of total larvae surviving, and larger larvae should also have an increased chance of survival. If these findings are consistent, then opaque egg masses may lead to higher fitness particularly in low nutrient conditions with more predators.

In my study, clear and opaque egg mass ratios varied tremendously with elevation; in the high elevation ponds, the proportion of clear egg masses was 18%, whereas the proportion of clear egg masses in low elevation ponds was 98%. These results suggest environmental variance is influencing the selection pressure for clear versus opaque clutches. However, I found that both water chemistry and predator communities varied between the high and low elevation sites, and it is possible that many other unmeasured environmental variables could be important too. The presence of predators, pH and NO_3^- appear important but as these data are correlative, this is not conclusive. An improvement to understanding how predator communities might shape distribution would be quantifying the abundance of each predator. Additionally, I only compared high and low elevation sites and more of an elevational gradient across North Carolina may help better understand how water chemistry and predator communities shape this polymorphism.

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Dep. Var.	Source	AIC	X^2	Model p
SPC	Elevation	-220.552	7.395	0.007
pH	Elevation	36.548	12.300	<0.001
NO_3^-	Elevation	53.596	0.075	0.784

Table 1. Differences in water chemistry parameters of ponds sampled in high (n = 11) versus low (n = 9) elevation ponds of North Carolina.

Source	Elevation	n	Mean	Std. Deviation
SPC	Low	9	51.53	25.26
SPC	High	11	121.00	69.50
pH	Low	9	5.66	0.70
pH	High	11	6.62	0.39
NO_3^-	Low	9	0.76	1.24
NO_3^-	High	11	0.98	1.13

Table 2. Means and standard deviations for water chemistry sampled in ponds in high (n = 11) versus low (n = 9) elevation ponds of North Carolina.

Dep. Var.	Source	AIC	X^2	Model p
% clear	SPC	-15.667	0.907	0.341
% clear	pH	-26.844	12.084	0.001
% clear	NO_3^-	-21.331	4.903	0.027

Table 3. Water chemistry predictors of clutch ratio of *A. maculatum* egg masses sampled in high elevation ponds of North Carolina (n = 11).

Dep. Var.	Source	AIC	X^2	Model p
% clear	SPC	-36.178	0.876	0.349
% clear	pH	-35.615	0.313	0.576
% clear	NO_3^-	-35.505	0.202	0.653

Table 4. Water chemistry predictors of clutch ratio of *A. maculatum* egg masses sampled in the low elevation ponds of North Carolina (n = 9).

Source	AIC	F value	Model p
Newt	-35.283	22.158	<0.001
Newt*Fish	-35.238	7.134	0.001
Caddisfly	-32.948	14.090	0.001
Crayfish*Newt	-32.598	8.104	0.001
Caddisfly*Crayfish	-32.442	8.802	0.002
Crayfish*Newt*Fish	-31.673	6.615	0.002
Caddisfly*Newt	-31.327	10.157	<0.001
Total Predators	-30.004	8.872	0.001
Caddisfly*Fish	-29.985	6.987	0.005
Caddisfly*Newt*Crayfish	-29.635	6.509	0.002
Caddisfly*Fish*Newt	-29.133	7.910	0.001
Newt*Fish*Caddisfly*Crayfish	-28.819	6.087	0.002
Wood Frog*Newt	-26.654	7.222	0.002
Crayfish	-25.242	1.068	0.314
Caddisfly*Wood Frog	-25.177	4.349	0.018
Wood Frog*Newt*Fish	-24.901	5.855	0.004
WF*Crayfish*Newt*Caddisfly	-24.556	4.258	0.011
Fish	-24.209	0.003	0.956
Wood Frog	-23.190	0.577	0.456

Table 5. Predator models predicting proportion of clear masses of *A. maculatum* in the high elevation ponds (n = 24) of North Carolina. The best fit models are in in bold.

Source	AIC	F value	Model p
Newt	-57.270	5.105	0.042
Marbled Salamander	- 57.098	7.340	0.018
Crayfish	-55.617	2.599	0.131
Marbled*Crayfish	-49.337	-2.707	0.131
Crayfish*Newt	-43.425	-1.598	0.246
Total Predators	-42.831	2.267	0.138

Table 6. Predator models predicting proportion of clear masses in the low elevation ponds (n = 15) of North Carolina. The best fit models are in in bold.

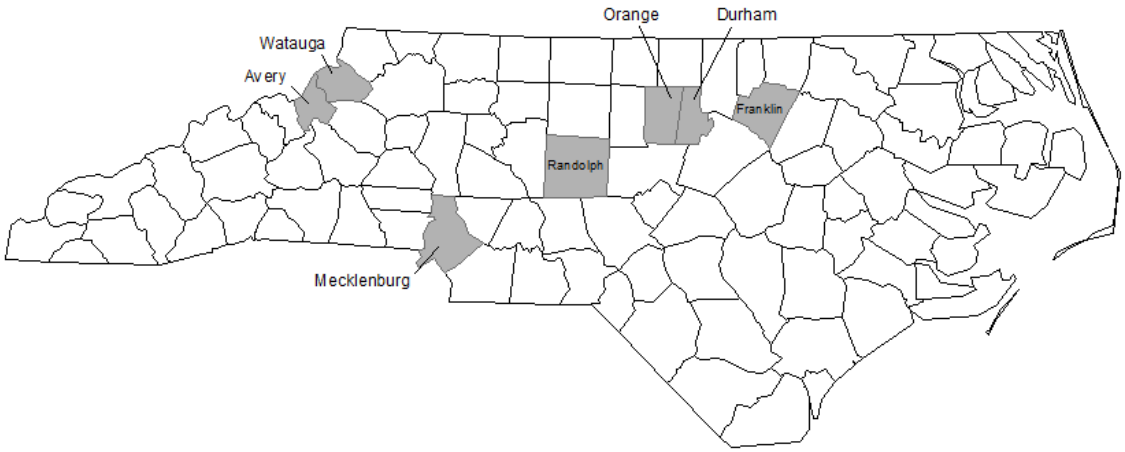

Figure 1. Data was collected in the highlighted and labeled counties of North Carolina. High elevation sites were within the counties of Avery and Watauga. Low elevation sites were within the counties of Durham, Franklin, Mecklenburg, Orange, and Randolph.

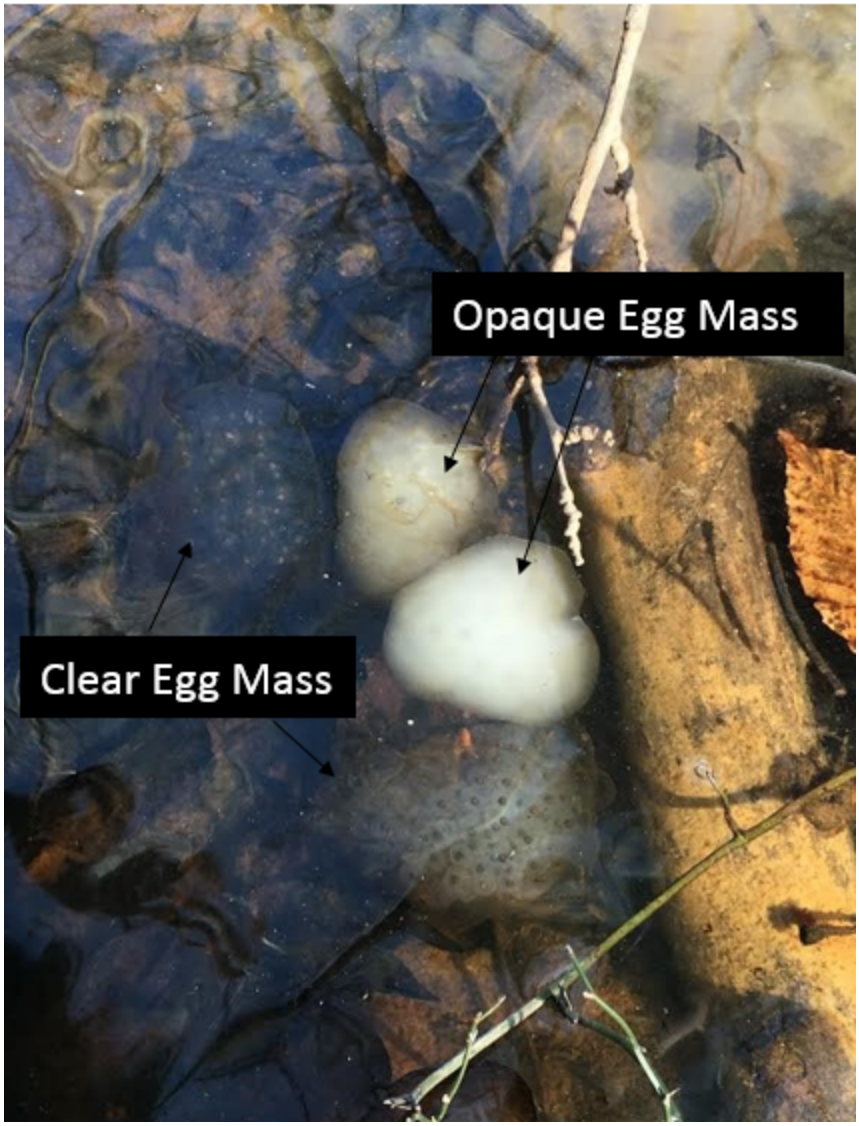

Figure 2. Representations of the clear and opaque dimorphism in *Ambystoma maculatum* egg masses.

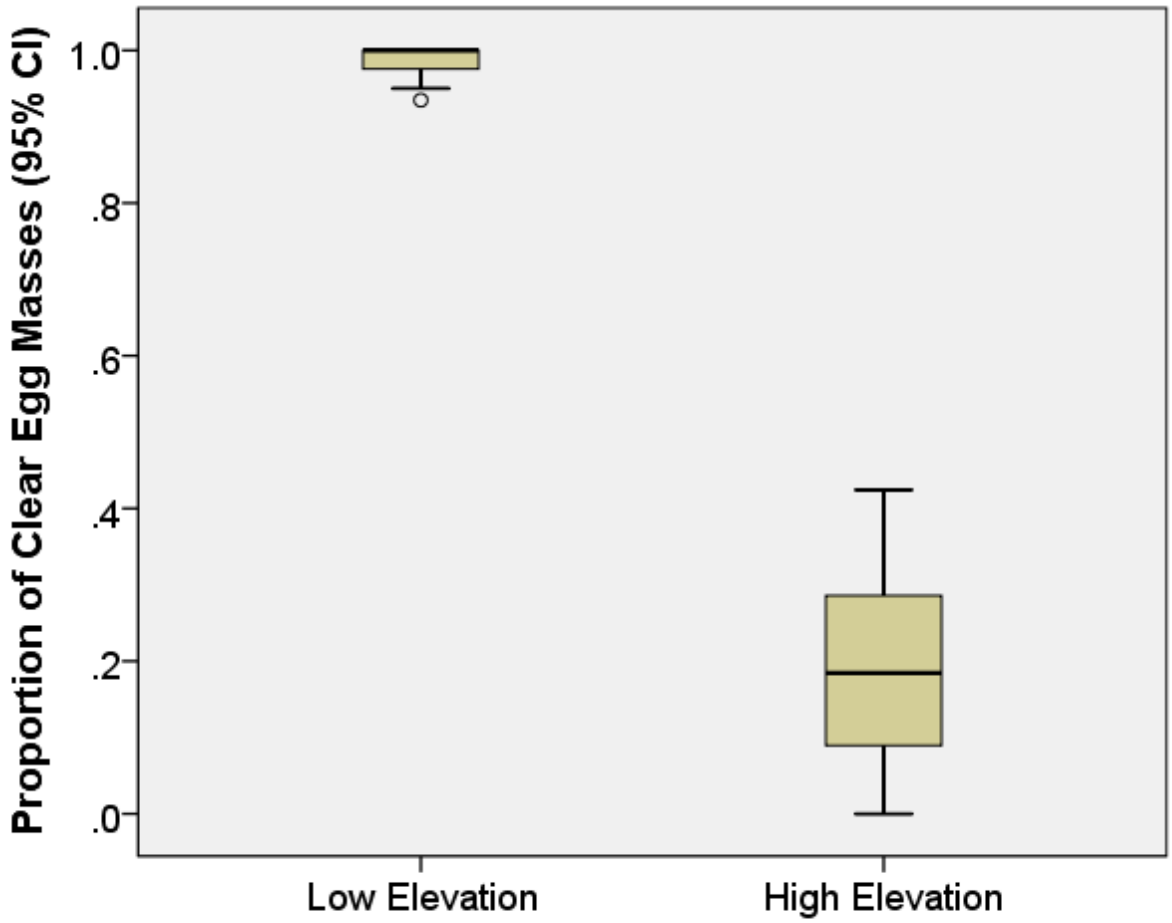

Figure 3. Comparison of *A. maculatum* egg mass ratio between high elevation (944-1061m) and low elevation (59-241m) field sites in North Carolina. Low elevations were associated with high proportions of clear egg masses (~98%); high elevations were associated with low proportions of clear egg masses (~18%). The line in the box represents the median, the box represents the 25th and 75th percentiles, and the whistlers represent the 10th and 90th percentiles.

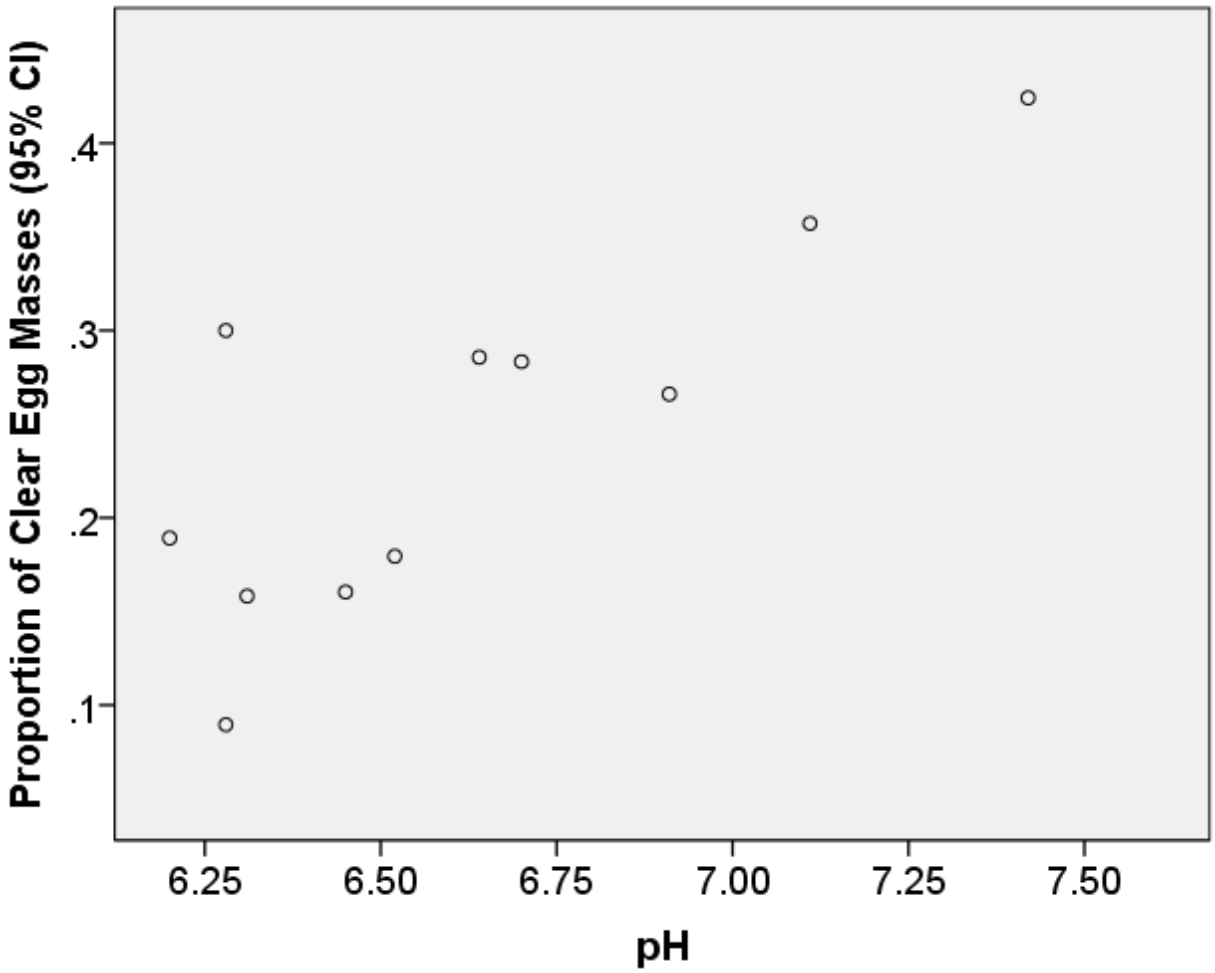

Figure 4. Relationship between water pH and proportion of *A. maculatum* clear egg masses in the high elevation ponds (n = 11) of North Carolina.

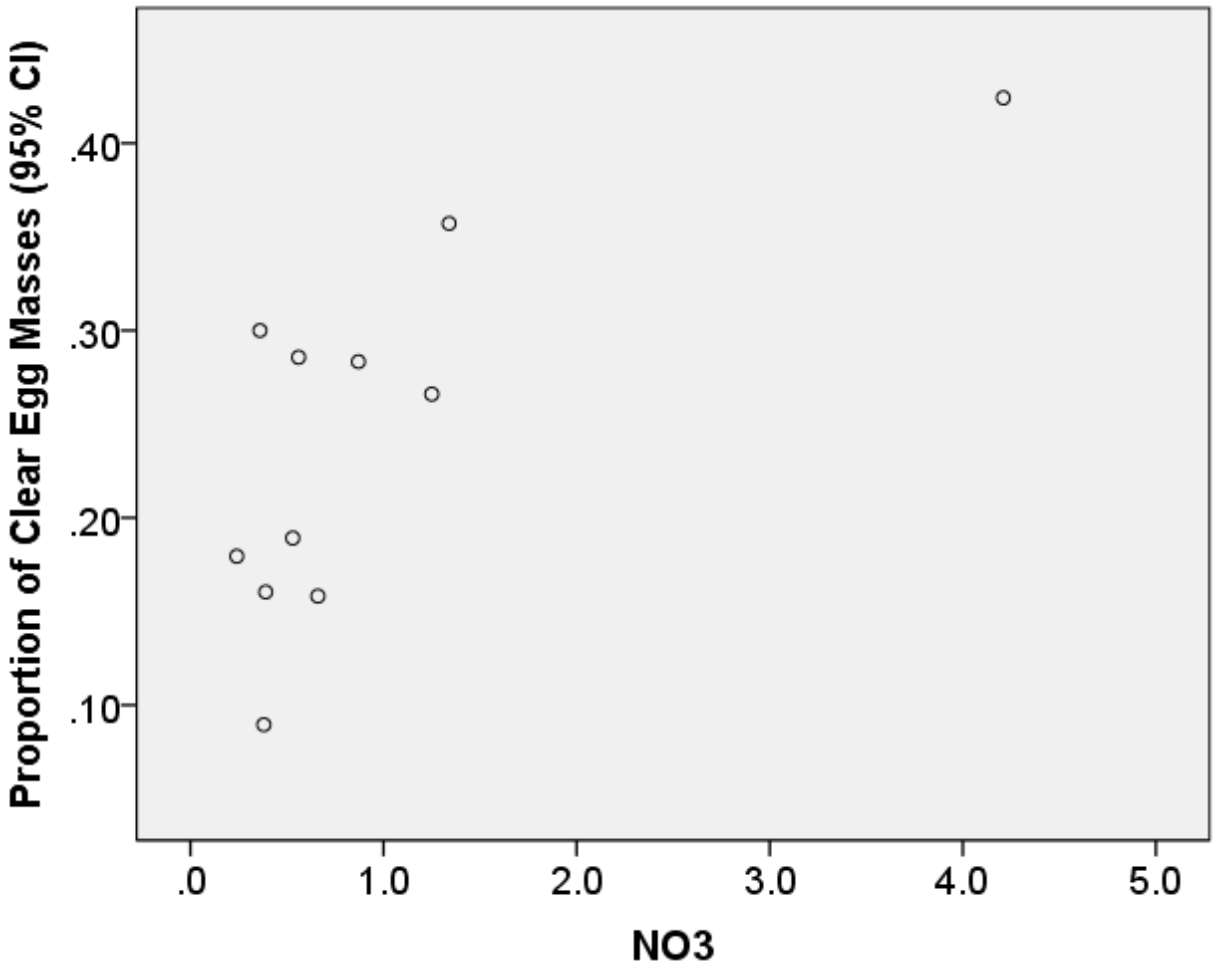

Figure 5. Relationship between dissolved nitrate (NO_3^-) and proportion of *A. maculatum* clear egg masses in the high elevation ponds (n = 11) of North Carolina.

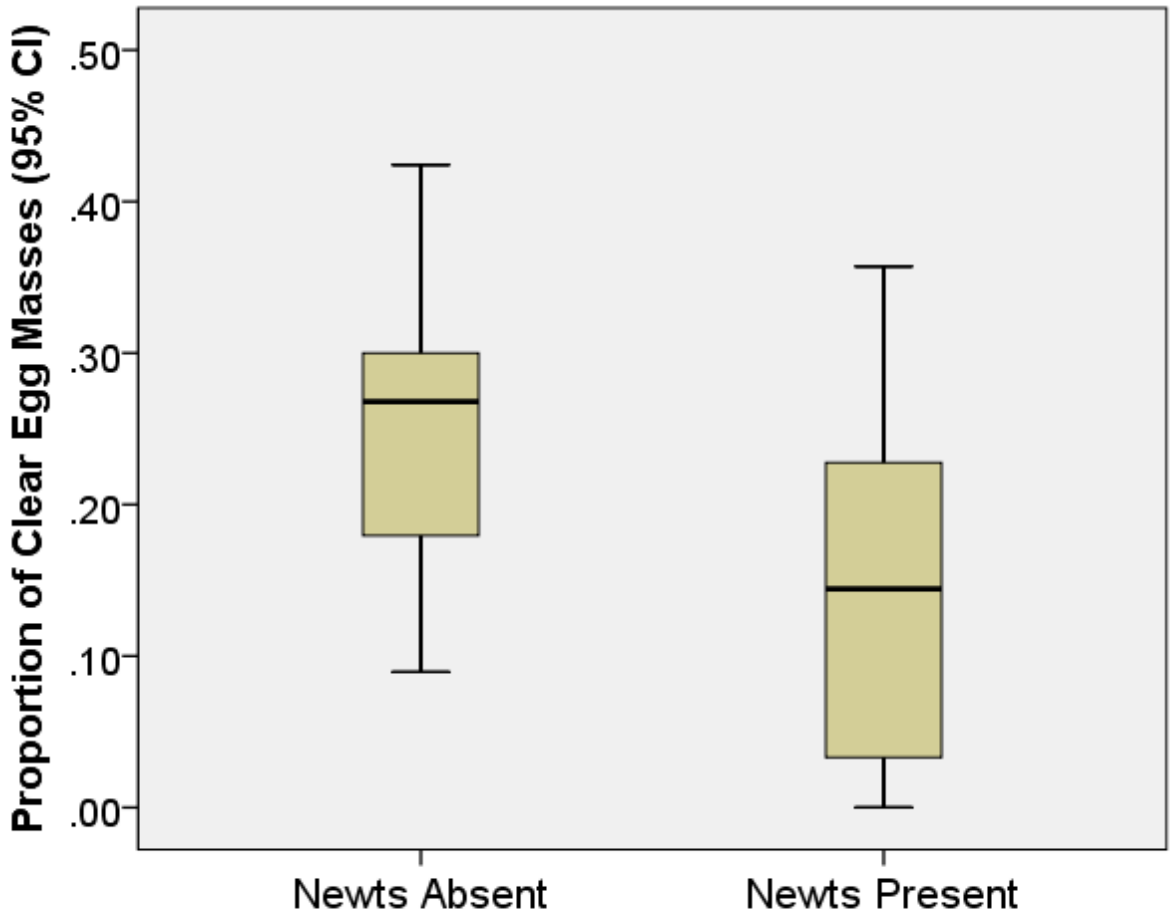

Figure 6. Comparison of *A. maculatum* egg mass ratio of ponds with and without newt (*Notopthalmus viridescens*) presence in high elevation ponds of North Carolina. The line in the box represents the median, the box represents the 25th and 75th percentiles, and the whistlers represent the 10th and 90th percentiles.

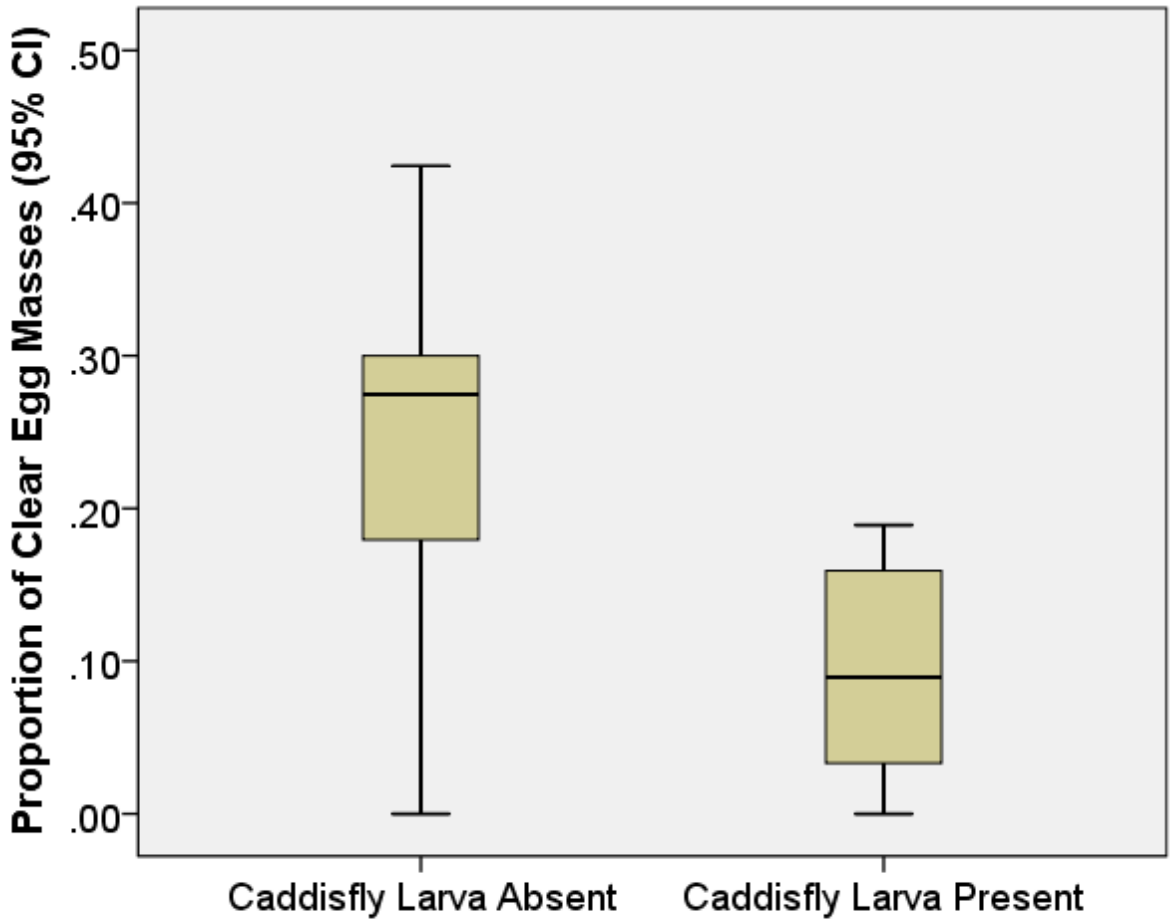

Figure 7. Comparison of *A. maculatum* egg mass ratio of ponds with and without caddisfly larvae in the high elevation ponds of North Carolina. The line in the box represents the median, the box represents the 25th and 75th percentiles, and the whistlers represent the 10th and 90th percentiles.

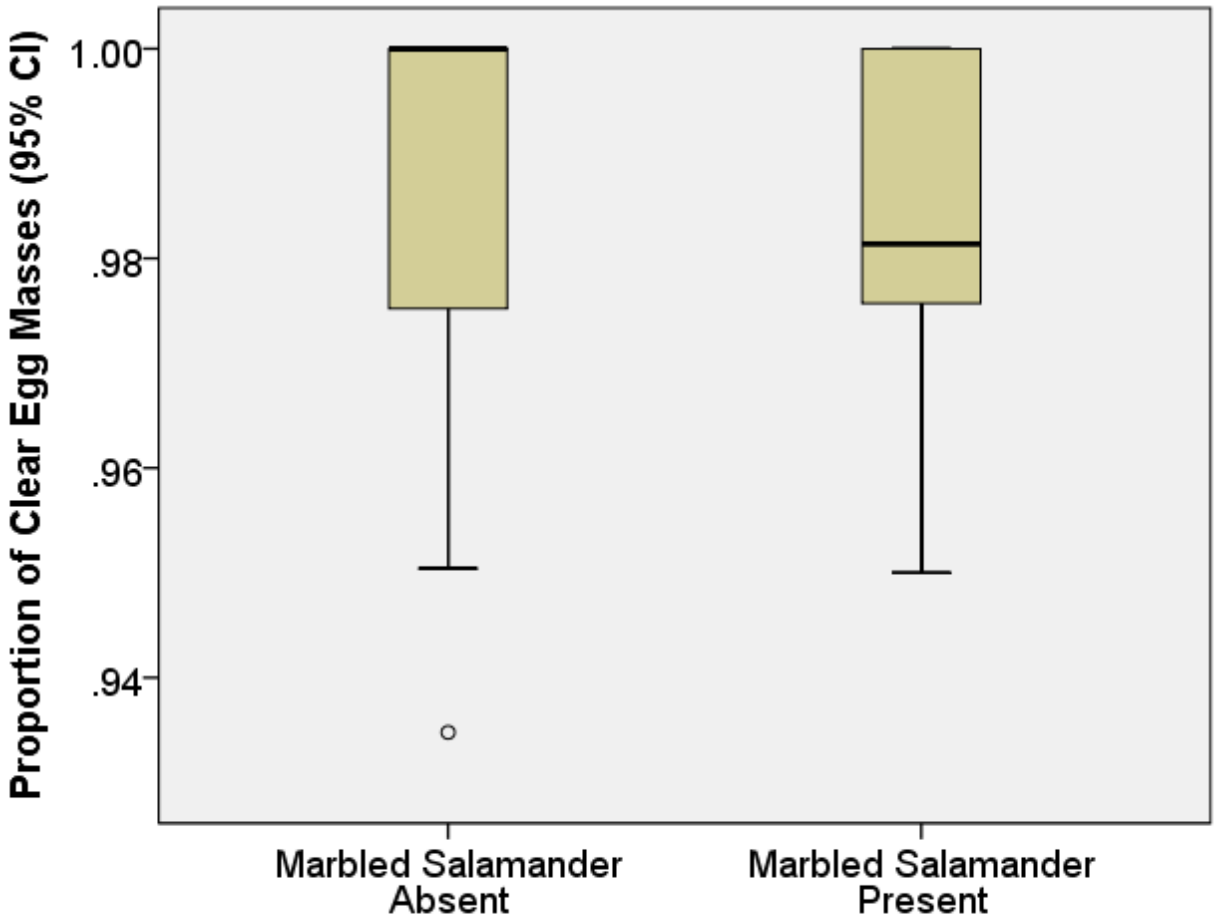

Figure 8. Comparison of *A. maculatum* egg mass ratio of ponds with and without marbled salamanders (*Ambystoma opacum*) in the low elevation ponds of North Carolina. The line in the box represents the median, the box represents the 25th and 75th percentiles, and the whistlers represent the 10th and 90th percentiles.

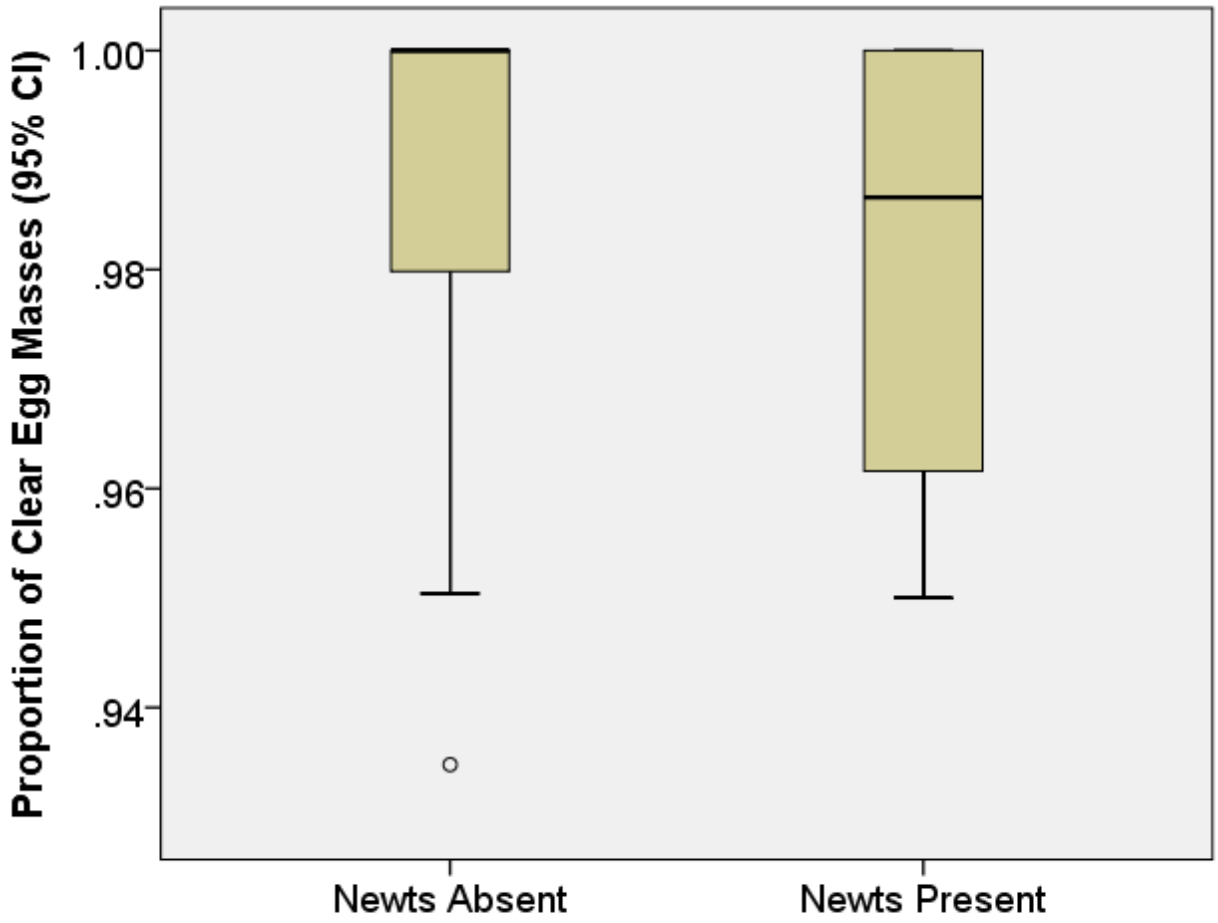

Figure 9. Comparison of *A. maculatum* egg mass ratio of ponds with and without eastern newts (*Notophthalmus viridescens*) in the low elevation ponds of North Carolina. The line in the box represents the median, the box represents the 25th and 75th percentiles, and the whistlers represent the 10th and 90th percentiles.